Avian Hosts of *Ixodes pacificus* (Acari: Ixodidae) and the Detection of *Borrelia burgdorferi* in Larvae Feeding on the Oregon Junco

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ABSTRACT Larval and nymphal western blacklegged tick, *Ixodes pacificus* Cooley & Kohls (Acari: Ixodidae), were collected from birds, rodents, and lizards at Quail Ridge Reserve located in Napa County in northwestern California. Species from three vertebrate classes were sampled simultaneously from two transects during two consecutive spring seasons. Feeding larval and nymphal ticks were removed and preserved for counting, examination and testing for the presence of *Borrelia burgdorferi* Johnson, Schmid, Hyde, Steigerwalt & Brenner. Mean infestations with *I. pacificus* subadults on lizards were 10.0, on birds 2.9, and on rodents 1.3. *I. pacificus* larvae (204) collected from 10 avian species and (215) collected from two rodent species were tested for the presence of *B. burgdorferi* s.s. via real-time polymerase chain reaction. Three *B. burgdorferi*-infected larvae were taken from two *Junco hyemalis* and two infected larvae from one *Neotoma fuscipes* Baird. This is the first reported detection of *B. burgdorferi* in larvae feeding on a bird in western North America.

KEY WORDS birds, rodents, lizards, *Ixodes pacificus*, *Borrelia burgdorferi*

Wild birds comprise a very large and extremely diverse taxonomic group with complex and still uncertain phylogenetic affinities. This group is collectively important to public health as many species can harbor and transport zoonotic pathogens and/or arthropod vectors of pathogens (Reed et al. 2003, Scott and Durden 2009, Scott et al. 2010). Individual bird species may differ considerably in their suitability as hosts for vectors and in their competency as pathogen reservoirs. Identifying the key avian species that contribute to the spread and maintenance of zoonotic disease is an essential part in the understanding of its epidemiology and in developing prevention strategies.

Lyme borreliosis, a rodent-borne zoonosis, is the most common vector-borne disease in the United States (CDC 2007). The etiologic agent, a spirochete *Borrelia burgdorferi* s.s. is the genospecies known to be pathogenic to humans in North America (Gray et al. 2002). The important vertebrate reservoirs for *B. burgdorferi* in North America are rodents. In the west the rodent reservoirs include several species, particularly the western gray squirrel, *Sciurus griseus* Ord; the dusky-footed woodrat, *Neotoma fuscipes;* the California kangaroo rat, *Dipodomys californicus* Merriam; and perhaps to a lesser extent several mouse species, including *Peromyscus boylii* (Shufeldt); *Peromyscus truei* (Shufeldt); and deer mouse, *Peromyscus maniculatus* (Wagner) (Lane and Brown 1991, Peavey and Lane 1995, Brown and Lane 1996, Lane et al. 2005). Despite the dominant role of rodents as reservoirs for Lyme borreliosis, birds are known to have a role as effective hosts and transporters of infected ticks and also as subordinate reservoirs in other parts of the world. For example, several bird species have been documented as important hosts of *Ixodes ricinus* (L.) subadults and also serve as reservoirs of Lyme borreliosis in Europe (Jaenson et al. 1994, Hubálek et al. 1996). In eastern North America, a variety of bird species act as hosts and transporters of the blacklegged tick *Ixodes scapularis* Say and several abundant species function as reservoirs for Lyme borreliosis. Four bird families, in particular, the Turdidae (thrushes), the Emberizidae (sparrows), the Troglodytidae (wrens), and the ground-nesting Parulidae (wood warblers) each contain species that are *B. burgdorferi* reservoirs and harbor live spirochetes of *B. burgdorferi* within their blood (Stafford et al. 1995, Durden et al. 2001, Ginsberg et al. 2005, Morshed et al. 2005, Scott and Durden 2009, Brinkerhoff et al.2009). In experimental infections, a high proportion of bird-fed infected larvae remain infectious after molting to nymphs (Anderson et al. 1990, Richter et al. 2000). Many of the same bird species in these families occur in California.

In the west, the role that birds play in the maintenance of *Ixodes pacificus* Cooley & Kohls (Acari: Ixodidae) and *Borrelia burgdorferi* has yet to be fully described (Eisen et al. 2004a). Previous investigations in northern California identified some avian species that function as good

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hosts for both subadult stages of *I. pacificus* and some individual birds with *B. burgdorferi* s.s. within their blood (Lane et al. 2006, Wright et al. 2006). As yet, it is not known whether any particular bird species infected with *B. burgdorferi* in the west can transmit the spirochetes to feeding *I. pacificus* larvae.

The aim of our investigation was to inventory, avian, mammal, and reptile hosts of larvae and nymphs of *I. pacificus* and to observe the intensity of host infestation on the Quail Ridge Reserve. In addition, we attempted to identify candidates for avian reservoirs by searching for *B. burgdorferi* spirochetes within avian-fed *I. pacificus* larvae and nymphs. Because *I. pacificus* larvae are very rarely infected through transovarial transmission of *B. burgdorferi* (Lane and Burgdorfer 1987, Schoeler and Lane 1993), their detection in blood fed larvae would probably indicate transmission either through feeding on an infected host or by cofeeding adjacent to infected nymphs (Brinkerhoff et al. 2009).

Materials and Methods

Collection Site. The Quail Ridge Reserve (QRR) is located in Napa County, CA, and situated on a peninsula on Lake Berryessa. The QRR (7,839 km^2 [1,937 acres]) is part of the Natural Reserve System, John Muir Institute of the Environment, and is maintained by the University of California at Davis. The QRR exists within the inner Coast Range at ≈1,200 feet in elevation. The climate at the QRR is Mediterranean, i.e., with hot, dry summers and cool, wet winters. The average annual rain fall is ≈70 cm (27.4 in.) (University of California Davis 2004). Our specific sample site on the peninsula is mesic woodland, situated near ephemeral creeks and shaded by slope aspect. The sample area consisted of two ≈150-m transects: one transect running east to west and the other transect running north to south. The forest is dominated by oaks (*Quercus* spp.) with abundant oak and gray pine, *Pinus sabiniana* Douglas ex D. Don, leaf litter. The dominant vegetation consists of interior live oak, *Quercus wislizenii* A. De Candolle; blue oak, *Quercus douglasii* Hook & Arn.; valley oak, *Quercus lobata* Nee; poison oak, *Toxicodendron diversilobum* (Torr. & Gray) Greene; gray pine; toyon, *Heteromeles arbutifolia* (Roemer); whiteleaf manzanita, *Arctostaphylos manzanita* Parry; and hairy ceanothus, *Ceanothus oliganthus* (Hooker).

Collection of Ticks. We collected host-seeking adults of *I. pacificus* from oak leaf litter or from low-growing vegetation along the up-slope side of trails and roads using a 1-m^2 flannel flag in February and March 2008 and 2009. These collections served as presamples to test for the presence of *B. burgdorferi* spirochetes. Adult *I. pacificus* were separated by gender and stored in a refrigerated humidity chamber (98% RH and 4°C) until tested.

Collection of Vertebrates. Lizards were collected by hand or by noosing along transects when observed on any field day from March through June 2008 and 2009. Each examined lizard was measured and sexed if possible. All lizard-feeding ticks were removed and stored in isopropyl alcohol for identification and enumeration but not for testing.

Mammals were trapped using National and Sherman live-traps baited with oats, *Avena sativa* L., and peanut butter. Traps were placed overnight along transects during two trapping events each month from March through June 2008 and 2009. Trapped mammals were anesthetized with diethyl ether, ear tagged, and aged, sexed and measured, before being released. Blood was collected via retro-orbital sinus by using a capillary tube or via cardiac puncture using needle and syringe. Duplicate thin blood smears were prepared from each rodent. Rodents were examined using a 30× diopter. All infesting ticks were removed with forceps, preserved in absolute ethyl alcohol, and held at 4°C for testing. Before testing each tick was examined for degree of blood engorgement and identification.

Birds were captured on two consecutive days twice each month from March through June in 2008 and 2009. Captures were accomplished using 15 mist nets (6 by 12 m, 38-mm mesh) placed along the established transects and operated for 4 h each day starting just after sunrise. Each bird species was banded using bands provided by U.S. Geological Survey (USGS) Bird Banding Laboratory in Laurel, MD; aged; and sexed if possible; and body conditions including fat deposition, molt, and wear were recorded. Morphological measurements were collected including a series of bill measurements, wing chord, tail, and weight. A small blood sample (<0.10 ml) was collected via jugular puncture using a 28-gauge needle and syringe. Duplicate thin blood smears were prepared from each bird. Birds were inspected in the field using either a compound microscope or head mounted diopter operated at 30–50×. All ticks were removed using forceps and preserved in absolute ethyl alcohol and held at 4°C for testing.

The birds described in this study were captured and handled in the field with permission from the USGS, Bird Banding Laboratory, under permit 22853. Rodents and birds were trapped with permission from California Department of Fish and Game scientific collecting permit SC-002994. This research was conducted in accordance with the Guidelines for the Capture, Handling and Care of Mammals as approved by the American Society of Mammalogists and the Guidelines for the use of Wild Birds in Research as approved by the Ornithological Council.

Identification of Ticks. All Ixodidae tick larvae collected from the QRR were identified using dichotomous keys provided by Webb et al. 1990 and Kleinjan and Lane 2008. Nymphs and adults of *I. pacificus* were identified using keys prepared by Furman and Loomis 1984 and Durden and Keirans 1996.

Detection of Spirochetes in Larval Ticks. The larval *I. pacificus* collected from birds in 2008 and 2009 were independently tested using two different molecular methods in two separate laboratories: Dr. Lane's laboratory at University of California at Berkeley and the Sacramento-Yolo MVCD laboratory (Sacramento County Public Health Laboratory) in Sacramento. Ad-

ditionally, DNA from any positive amplicons was sequenced in Dr. Janet Foley's laboratory at University of California, Davis. The larval *I. pacificus* collected from rodents in 2008 and 2009 were all tested in the Sacramento-Yolo MVCD laboratory.

Extraction Procedure. In the Lane laboratory, larval ticks collected from birds in 2008 were triturated individually with sterile pestles in micro-centrifuge tubes. Total DNA was purified using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. After adding 180 μl of ATL buffer and 20 μl of proteinase K, samples were lysed overnight at 56°C. Next, 200 μl of AL buffer was added, and the resultant mixture was incubated at 70°C for 10 min. The final steps of the DNA then was eluted with 100 μl of elution buffer and stored at −20°C.

In the Sac-Yolo MVCD laboratory, larval ticks collected from birds in 2009 were placed into a 5-ml polycarbonate vial with three 5-mm glass beads. Then, 150 μl of NucPrep digestion buffer and 50 μl of NucPrep proteinase K solution were added to each individual sample. The samples were pulverized for 30 min in an 8000D Spex CertiPrep Mixer Mill (Spex CertiPrep, Metuchen, NJ) to digest tissue and release the nucleic acid. A heat-killed positive control of *B. burgdorferi* cellular antigen (Klp 50-97-91) and molecular grade water was used for a negative control during each extraction. Total DNA was purified using the NucPrep Chemistry: isolation of Genomic DNA from Animal and Plant Tissue (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. The DNA was extracted using the ABI 6100 Nucleic Acid Prep Station following protocols listed above. Finally, the DNA was eluted with 100 μl of NucPrep DNA Elution Solution 1 and 100 μl of NucPrep DNA Elution Solution 2. All samples were stored at −20°C.

PCR Assays. In the Lane laboratory, the detection of *B. burgdorferi* s.l. DNA was attempted by targeting the 5S-23S rRNA region in a nested PCR assay, as described previously (Girard et al. 2009), with minor modifications. Reaction conditions included 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s for outer primers (58°C for internal primers), and extension at 72°C for 1 min. Multiple negative and positive controls (deonized water and *B. burgdorferi* isolate CA4, respectively) were included with each run. The amplicons were visualized on an ethidium bromide-stained 1.5% agarose gel.

In the Sac-Yolo MVCD laboratory, the extracted DNA samples were tested using real-time PCR to detect *B. burgdorferi* s.s. through the amplification of the FL8 flagellum gene of the *B. burgdorferi* s.s. genome. The primers and probe set used to test the samples were designed by Marin-Sonoma MVCD (Heilig et al. 2010) and are modifications from *B. burgdorferi* (GeHo) *fla* gene for flagella filament 41-kDa core protein (GenBank accession X15660) originally by Pahl et al.1999. The sequences used are as follows: forward primer, 5′-CAAACCAAGATGAAGCTATTGCTGTA-3′; reverse primer, 5′-CTTCCTGTTGAACACCCTCTTGAA-3′; and MGB probe, 5′-FAM-CAGCCTGAGCAGTTGA-MGB-3′.

PCR was performed on an ABI 7500 real-time PCR system using TaqMan Chemistry. The reaction conditions include one repetition at 95°C for 10 min and 45 repetitions at 95°C for 15 s, 60°C for 1 min. Positive samples are determined by threshold cycle (Ct) values with all samples having a threshold cycle value of 40 or below considered positive. The lower the Ct values the greater amount of spirochete DNA present in the extracted sample. An established Ct value of 40 or below is conservatively considered to be *B. burgdorferi* positive.

Spirochetes in Blood. Blood smears from birds with *B. burgdorferi*-infected ticks were tested for spirochetes by using both real-time PCR and an indirect immunofluorescent assay (IFA) at the Sac-Yolo laboratory. Half of each blood smear collected from a bird with an infected tick was scraped from both replicate slides by using a razor blade before staining and tested by PCR and IFA.

Blood smears were allowed to dry in the field and held at 4°C before fixing with absolute methanol (25°C) for 25 ± 10 min. Smears were examined via IFA by using a polyclonal anti-Osp A and Osp C antibody specific to *B. burgdorferi* s.l. with fluorescein isothiocyanate anti-mouse antibody as the fluorescent label. Each exposed smear was allowed to incubate with the anti-Osp A and Osp C antibody diluted in phosphate-buffered saline (PBS) and then rinsed in PBS. Fluorescently labeled anti-mouse antibody was added to each smear and allowed to incubate for 60 min and rinsed again using PBS. Slide stains were viewed through 80% glycerol and a cover-glass by using an epifluorescent microscope.

Results

In 2008, two of 150 (1.3%) host-seeking adult *I. pacificus* were infected with *Borrelia burgdorferi* s.s. In 2009, one of 130 (0.8%) ticks collected from the same locations was infected. The abundance of host-seeking *I. pacificus* along transects was approximated at two ticks per minute of flagging by using the standard m^2 flannel cloth. Only one other host-seeking tick, the Pacific Coast tick, *Dermacentor occidentalis* Marx, was collected with *I. pacificus* at the site.

Small Vertebrate Infestation. In total, 52 lizards of three species were captured and examined for attached ticks. The mean infestation, percentage of infestation prevalence and larva-to-nymph ratios (L:N) for the alligator lizard, the fence lizard, and the western skink, *Plestiodon* (formally *Eumeces*) *skiltonianus*, are shown in Table 1. Six inspections of the ring-necked snake, *Diadophis punctatus*, yielded no ticks.

In total, 182 inspections were made on 120 new and 62 recaptured mammals from five species. The Pinyon mouse, *Peromyscus truei;* the brush mouse, *Peromyscus boylii* (Shufeldt); and the dusky-footed woodrat, *Neotoma fuscipes*, were infested almost exclusively with larval *I. pacificus* subadults. A single *I. pacificus* nymph was collected from *N. fuscipes.* Mean infestations (M) and percentage of prevalence are shown in Table 2. One individual deer mouse and one ornate shrew,

Table 1. Lizard species examined with *I. pacificus* subadult ratio, mean infestation, and percentage of prevalence for the Quail Ridge Reserve, Napa Co., CA, 2008 and 2009

Scientific name	Common name	Lizards examined	No. *I. pacificus*	L:N ratio	Mean	Prevalence
Elgaria multicarinata	Southern alligator lizard	8	162	3:2	20.50	100
Sceloporus occidentalis	Western fence lizard	22	323	2:3	14.68	95
Plestiodon skiltonianus	Western skink	22	35	4:1	1.59	50

Sorex ornatus, that were examined were without ticks. Three nymphs of the nidicolous or nest-inhabiting tick *Ixodes spinipalpis* Hadwen & Nuttall were collected from three individuals of *N. fuscipes* from a single sample set on 28 March 2008. Two rodent species, the dusky-footed woodrat and the brush mouse, also were parasitized by both larval and nymphal stages of the Pacific Coast tick.

During the same period, 222 inspections in total were made from 193 new and 29 recaptured birds from 19 species. Of the 12 avian species infested with *I. pacificus*, nine species were found infested with both larval and nymphal stages of *I. pacificus* and two species, the American robin, *Turdus migratorius* (L.) ($n = 2$) and the Hutton's vireo, *Vireo huttoni* Cassin ($n = 2$), were infested with only the nymphal stage. Seven species of bird were without infestations of *I. pacificus* (Table 2).

Larvae Feeding on Birds and Rodents. In total, 204 *I. pacificus* larvae were removed from 10 bird species and 215 *I. pacificus* larvae from three rodent species and tested via PCR for the presence of *Borrelia burgdorferi* s.s. Remaining larvae of *I. pacificus* from avian and rodent hosts were not tested (Table 2).

Avian species that forage in leaf litter and or nest on the ground, including the Oregon junco, orange-crowned warbler, house wren, and both towhee species are infested with 3 times as many larvae as nymphs (387 larvae:130 nymphs), whereas species that forage on tree bark and in the canopy to include the brown creeper, black-headed grosbeak, purple finch, and oak titmouse have 3 times as many nymphs as larvae (27 larvae:86 nymphs).

Individual larval ticks were omitted from testing if all the diagnostic characters could not be clearly observed typically due to specimen damage. In the case of larvae from the oak titmouse 15 of the 16 were apparently dead and dried and frequently missing legs, palps, or both.

PCR. From the rodent-fed *I. pacificus* larvae, two positive larvae were detected in 2008 and no positive larvae were detected in 2009. The two *B. burgdorferi* s.s.-positive larvae were collected from a single adult male dusky-footed woodrat sampled on 11 April. The two positive rodent-feeding larvae represent 1.9% of the overall rodent-feeding larvae tested for 2008, and a 2.2% infection of woodrat-feeding larvae. The dusky-

Table 2. Rodent and bird species examined with *I. pacificus* larval and nymphal numbers and larvae tested, mean infestation, and percentage of infestation prevalence for the Quail Ridge Reserve, Napa Co., CA, 2008 and 2009

Scientific name	Common name	Hosts examined	Larvae (tested)	Nymphs	Total	Mean	% infestation
Class Mammalia							
Neotoma fuscipes	Dusky-footed woodrat	43	103 (99)	1	104	2.42	65
Peromyscus boylii	Brush mouse	132	117 (114)	0	117	0.89	45
Peromyscus truei	Pinyon mouse	5	8 (2)	0	8	0.60	60
Peromyscus maniculatus	Deer mouse	1	0	0	0	0	0
Class Aves							
Junco hyemalis	Oregon junco	89	285 (152)	107	392	4.38	75
Pipilo crissalis	California towhee	2	15 (5)	6	21	10.00	100
Pipilo maculata	Spotted towhee	6	19 (10)	3	22	3.83	67
Carduelis psaltria	Lesser goldfinch	8	1 (1)	0	1	0.12	12
Carpodacus purpureus	Purple finch	28	9 (5)	13	22	0.86	50
Pheucticus melanocephalus	Black-headed grosbeak	4	1 (1)	9	10	2.75	50
Piranga ludoviciana	Western tanager	2	0	0	0	0	0
Molothrus ater	Brown-headed cowbird	2	0	0	0	0	0
Vermivora celata	Orange-crowned warbler	20	26 (10)	3	29	1.30	55
Vireo cassinii	Cassin's vireo	4	0	0	0	0	0
Vireo huttoni	Hutton's vireo	2	0	1	1	0.50	50
Catharus guttatus	Hermit thrush	1	0	0	0	0	0
Turdus migratorius	American robin	2	0	4	4	2.00	100
Trogolodytes aedon	House wren	2	42 (18)	11	53	25.50	100
Certhia americana	Brown creeper	4	1 (1)	41	42	10.25	75
Baeolophus inornatus	Oak titmouse	25	16 (1)	23	39	1.52	68
Empidonax difficilis	Pacific-slope flycatcher	14	0	0	0	0	0
Melanerpes formicivorus	Acorn woodpecker	6	0	0	0	0	0
Drycopus pileatus	Pileated woodpecker	1	0	0	0	0	0

footed woodrat with two infected larvae was additionally infested with two uninfected larvae.

From the bird fed *I. pacificus* larvae, three *B. burgdorferi* s.s.-positive larvae were identified from two Oregon juncos. Two of the *B. burgdorferi* s.s.-positive larvae and one positive *B. burgdorferi* s.l. were collected from a hatch-year (immature) junco captured on 21 May 2009 and a third *B. burgdorferi* s.s.-positive larva was collected from an adult male junco sampled on 12 June 2009. The three positive bird-feeding larvae represent ≈2.3% of the overall bird-feeding larvae tested for 2009, and a 2.6% infection of junco-fed larvae. The Oregon junco with infected larvae was additionally infested with five uninfected larvae and three uninfected nymphs of *I. pacificus.* A second Oregon junco with a single infected larva also was infested with three nymphs of *I. pacificus* one of which was positive for *B. burgdorferi* s.s. The DNA of positive amplicons generated from the PCR assays was subsequently sequenced and supports the sensu stricto finding.

Spirochetes were not detected by IFA or PCR within the blood smears made from the two Oregon juncos with infected ticks. Other blood samples from birds and rodents without infected ticks remain untested at this time due to operating expense.

Discussion

California has a diverse array of habitats that create a complex mosaic of conditions that support the 3-yr, three host life cycle of the western blacklegged tick, *I. pacificus* (Padgett and Lane 2001) and a great variety (108 vertebrate species) of bird, mammal and lizard hosts (Furman and Loomis 1984, and Castro and Wright 2007).

In California, a variety of rodents function as hosts for subadult stages of *I. pacificus* and as reservoirs for the Lyme disease spirochete, *B. burgdorferi.* Evidence indicates that the principal rodent reservoir of *B. burgdorferi* is the western gray squirrel, *S. griseus* (Lane et al. 2005, Salkeld et al. 2008 and Salkeld and Lane 2010).

In contrast, a variety of avian species in California seem to host substantial numbers of immature *I. pacificus* (Wright et al. 2003b), but remain relatively underrepresented and infrequently included in studies of hosts and reservoirs for Lyme disease spirochetes (Eisen et al. 2008 and Salkeld and Lane 2010). In northern California, bird species in the thrush, sparrow and wren families were found infested with both larvae and nymphs of *I. pacificus* (Wright et al. 2000 and 2006). Some individuals, a hermit thrush, *Catharus guttatus* and a wild turkey, *Meleagris gallopavo*, has been identified infected with spirochetes of *B. burgdorferi* s.s. (Lane et al. 2006 and Wright et al. 2006).

The Quail Ridge Reserve stands rather typically as a Californian interior coast foothill location with low levels of adult *I. pacificus* infected with *B. burgdorferi.* Our specific study site at QRR found three species of lizard infested with both subadult stages of *I. pacificus.* The southern alligator lizard, *Elgaria multicarinata*, had the heaviest mean infestations followed by the western fence lizard, *Sceloporus occidentalis*, and lastly the western skink, *Plestiodon skiltonianus.* The two species of lizard with the heaviest infestations, the alligator lizard and the fence lizard are both *Borrelia*-refractive, incompetent reservoirs and suppress infection in previously infected feeding ticks (Lane and Quistad 1998, Wright et al. 1998). Because the subadult stages of *I. pacificus* have a preference for refractive lizards as hosts, the many lizard-fed ticks can be discounted as contributing to the *B. burgdorferi* infected pool. This leaves a smaller cohort of ticks feeding on the reservoir competent rodents and perhaps some species of birds to maintain local spirochete transmission.

The detection of *B. burgdorferi* infected *Neotoma*-feeding larvae from a Lyme borreliosis endemic location was anticipated based on the previous establishment of woodrats as spirochete competent reservoirs (Brown and Lane 1992). The western gray squirrel, *S. griseus* is a common rodent at QRR and the important *B. burgdorferi* reservoir in the west and therefore likely makes a contribution to tick infection at QRR but was not targeted for sampling during this study.

Our woodrat and mouse infestation results reflect other published findings for mean infestations with *I. pacificus* in northern California locations (Lane and Loye 1991, Wright et al. 2000, Casher et al. 2002, and Eisen et al. 2004b). Three species of rodent, two *Peromyscus* mice and the *Neotoma* woodrat, (180 individuals) were infested almost exclusively by larvae of *I. pacificus* suggesting either a larval feeding preference or a nymphal feeding aversion for rodents or perhaps rodent occupation of only larval host-seeking microhabitats at the QRR.

Some species of birds examined in this study seem to be good hosts for subadults of *I. pacificus.* Several avian species that are ground-dwelling and/or leaf litter-foraging specialists are infested with a greater proportion of larvae than nymphs, whereas bird species that specialize on tree bark and tree limbs are infested with a greater proportion of nymphs than larvae of *I. pacificus.* These results conform to those of Slowik and Lane 2001, Lane et al. 2007 and Lane et al. 2009, which indicate that a proportion of the nymphal population may climb trunks of trees or drop as blood fed larvae from arboreal hosts, molt and quest as nymphs within the associated moist microclimates. Our inspections of ground-foraging Oregon juncos found infestations with nearly three times as many larvae as nymphs, whereas bark forage specialists, such as brown creepers, were infested almost exclusively by nymphs of *I. pacificus.*

Birds with the heaviest infestations of *I. pacificus* at the QRR location are common year-round residents, such as the brown creeper and the oak titmouse, or spring and summer nesting residents and winter migrants, such as the Oregon junco and the purple finch. Infested migratory birds not only contribute as supportive hosts but likely also transport *I. pacificus* and perhaps *B. burgdorferi* to new and isolated habitats as

apparently occurred in the isolated Sutter Buttes (Wright et al. 2003a).

A commonly observed species in oak woodland habitats is the oak titmouse, *Baeolophus inornatus.* This species was the third most commonly encountered species in our mist nets with a relatively high *I. pacificus* mean infestation. Upon examination of this species, it was frequently noted that the attached ticks were dead and dried. This same phenomenon was observed at our other surveyed sites with abundant oak titmice such as on the Sutter Buttes and at Clear Lake State Park (unpublished observations). The titmice may be exhibiting an immune response that is detrimental to the attached ticks. This conjectured immune action could eliminate the titmouse as a good supportive host for *I. pacificus* subadults and also as a reservoir for spirochetes. In these habitats, an incompetent host such as the oak titmouse may reduce the number of ticks feeding on the reservoir competent western gray squirrel and thus reduce overall tick infections. Observations such as this support the need for further investigations of avian host competency to more fully understand the ecology of *I. pacificus* and Lyme borreliosis in northern California.

Our most abundantly sampled avian species the Oregon junco (Emberizidae) had infestations of over four ticks per bird and may support an important portion of the *I. pacificus* subadult population at QRR. One of the most widespread and abundant birds in North America, the Oregon junco breed and nest in conifer and deciduous forests in the Coast Range, Cascade and Sierra Nevada foothills of California, but especially in oak woodlands. The Oregon junco preferentially nests on the ground and forages by scratching in leaf litter and within the crevices of tree trunks for insects and seeds. This ground based nesting and mixed microhabitat forage behavior puts this species in close proximity to both host-seeking subadults of *I. pacificus* and therefore in a particularly good position as an important host for *I. pacificus.*

An infection with *B. burgdorferi* of at least one Oregon junco is a plausible supposition based on the detection of spirochetes in three feeding *I. pacificus* larvae. The likelihood that all three of these larvae were infected transovarially is very low (Schoeler and Lane 1993). However, the blood tested by both IFA and PCR from these birds were negative for spirochetes. It is possible that the spirochete density in the blood of infected juncos is below the detection limit of these tests or was missed due to a heterogeneous distribution of spirochetes. Feeding tick larvae concentrate host blood and perhaps spirochetes, bringing them to detectible levels.

Another possibility exists that an infected co-feeding nymph infected these larva. A subdermal feeding pocket created by a feeding infected nymph may concentrate spirochetes for days before their dissemination and may function as a source for infection of cofeeding larvae on birds (Stafford et al. 1995, Patrican 1997). Both juncos with infected larvae were also concurrently feed upon by nymphs. The junco with three infected larvae also was infested with three nymphs that all tested negative for spirochetes. The second junco with one infected larvae was also infested with three nymphs two of which were negative and one that was infected with *B. burgdorferi.* This combination of results leaves the nymphal cofeeding infection possibility somewhat ambiguous for at least one of the juncos, although an infected nymph may have recently detached after feeding to repletion. In either way, direct larval infection or infection via a cofeeding nymph, the Oregon junco probably played a role in the infection of *I. pacificus* larvae with *B. burgdorferi* at this coastal foothill site.

The Oregon junco, due to its high *I. pacificus* infestation and local abundance at the Hopland Field Station in the northern California Coast Range was considered a good host and a candidate reservoir species worthy of further investigation (Eisen et al. 2004a). In eastern North America, an Oregon junco was collected with *B. burgdorferi* s.l.-infected *I. scapularis*, signifying that they are capable of not only spreading ticks but also Lyme disease (Scott et al. 2010). The current study offers a first report of *B. burgdorferi* s.s. detection and probable infection of *I. pacificus* larvae feeding on the Oregon junco in western North America. In addition, we put forward the need for further investigations in the field and laboratory of the Oregon junco and other ground- and tree-foraging birds that may be acting as subordinate reservoirs infecting *I. pacificus* with *B. burgdorferi* in the west.

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